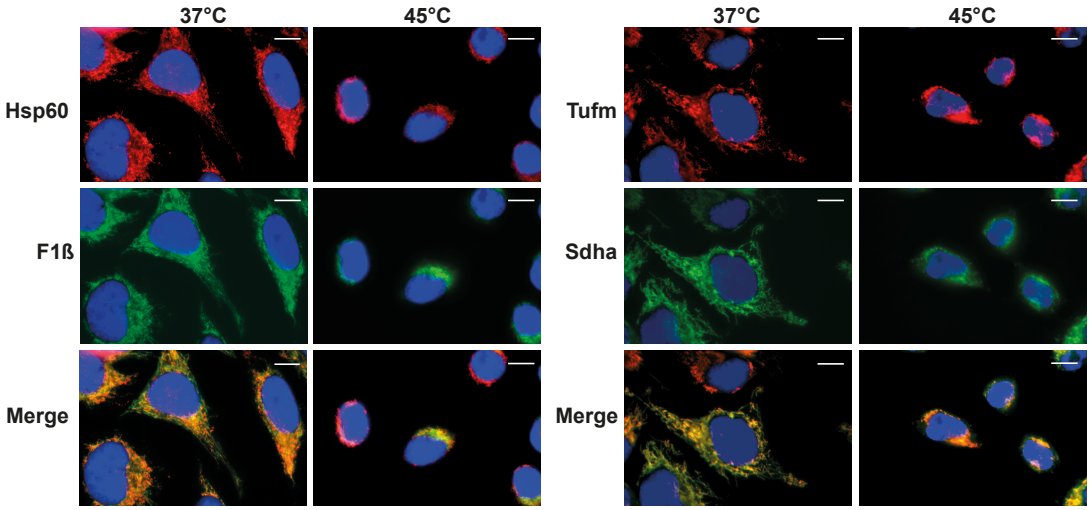

Fig. S1. Mitochondrial morphology after heat stress.
HeLa wild-type cells were treated for 2 h at indicated temperatures and analyzed by indirect immunofluorescence microscopy using antibodies against the mitochondrial proteins Hsp60, Tufm, Sdha, and F_1ß as indicated. Scale bar: 10 µm.

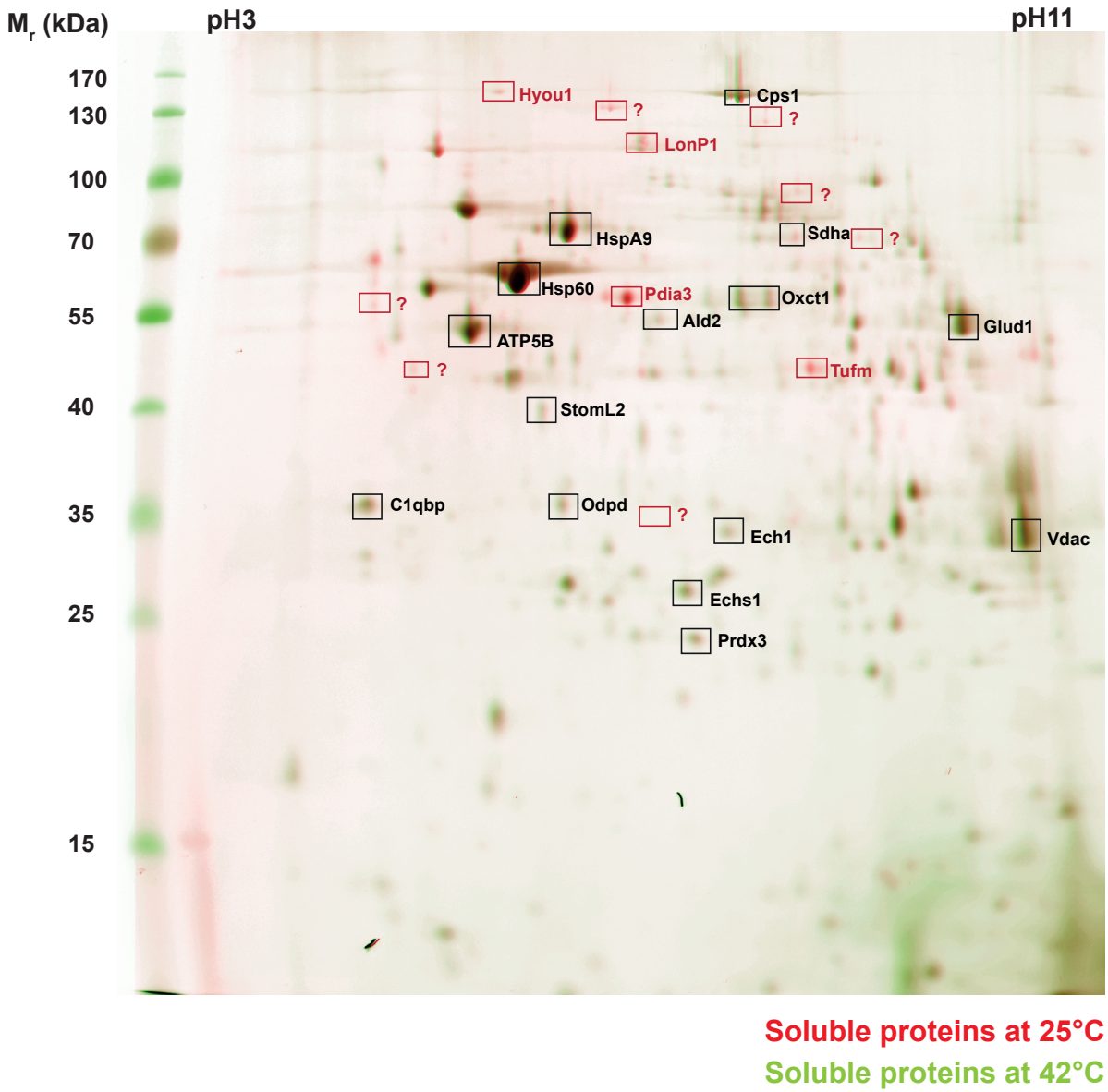

Fig. S2. 2D differential gel electrophoresis (DIGE) of heat-treated mitochondria. Isolated mitochondria were incubated for 20 min at 25°C or 42°C and soluble proteins were separated from aggregated proteins by differential centrifugation (125,000 xg, 40 min). The soluble protein fractions of control and stress samples as well as a standard were labeled with different fluorescent dyes and analyzed on one 2D gel (one representative gel of six analyzed experiments is shown). The picture represents an overlay of the soluble proteome of the control sample (red, 25°C) with the soluble proteome after heat stress (green; 42°C). Protein spots identified via mass spectrometry are indicated. Highlighted in red are proteins exhibiting a significant change in abundance.

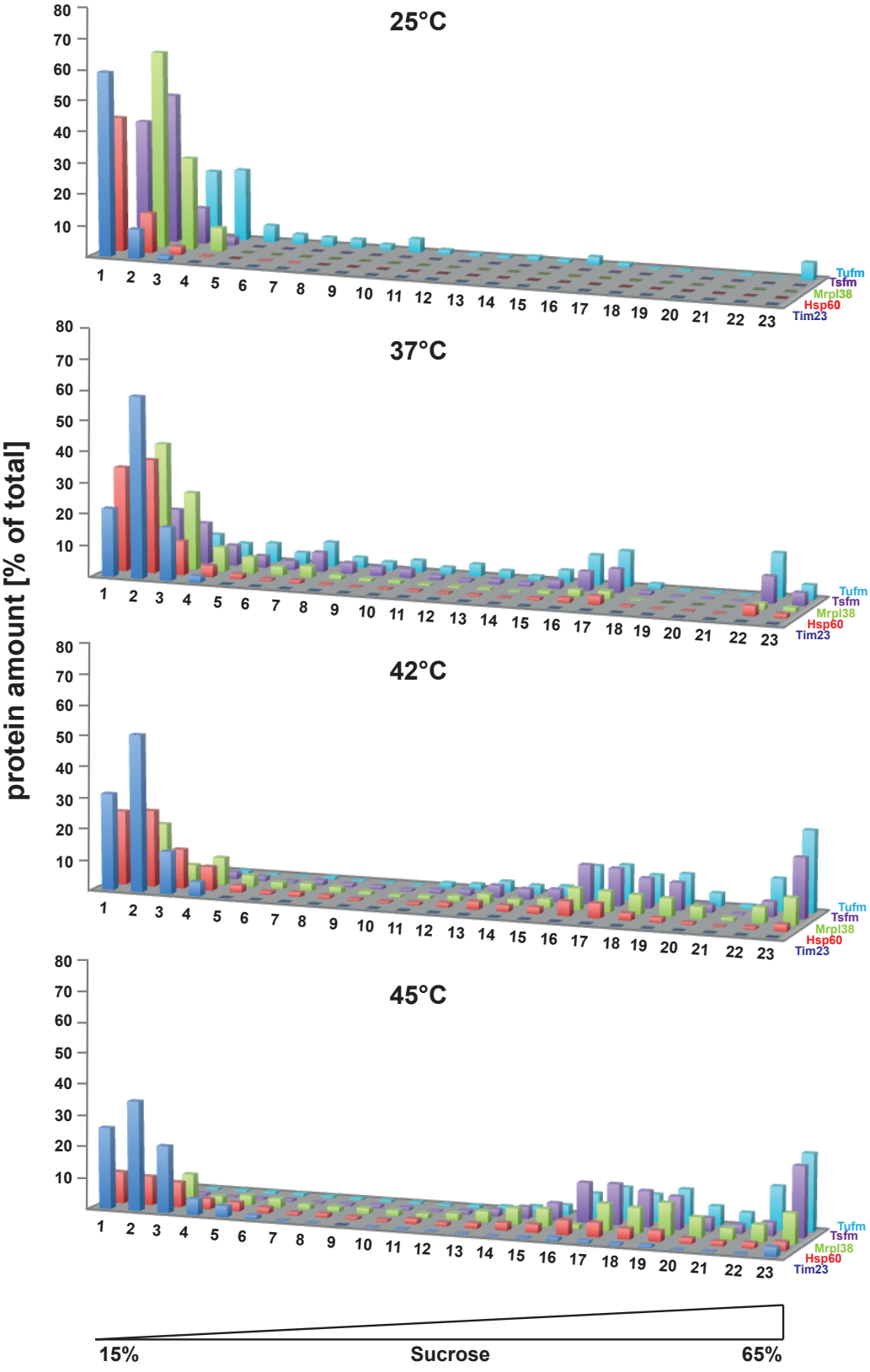

Fig. S3. Analysis of aggregates after heat stress by sucrose density gradient centrifugation. After 20 min incubation at indicated temperatures, mitochondria were lysed with 1% digitonin and proteins were separated by centrifugation (200,000 xg, 1 h, 4°C) according to their density on a sucrose gradient (15%–65% sucrose). The individual fractions of the gradient (fraction 1–23) were analyzed by SDS–PAGE and Western blot. Shown are quantifications of the immunodecoration signals for the proteins Tim23 (blue), Hsp60 (red), Mrpl38 (green), Tsfm (purple) and Tufm (turquois). The total protein was calculated by the sum of the signals of all fractions for every protein and set to 100%.

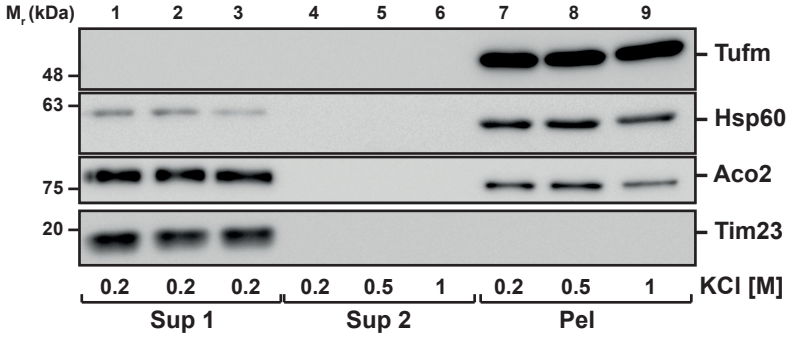

Fig. S4. Salt treatment of protein pellet fraction to assess ribosome association.
Isolated mitochondria were heat stressed at 42°C for 20 min and subsequently lysed in buffer containing low salt concentration (0.2 M KCl). Soluble proteins (Sup 1) and aggregates were separated by centrifugation and pellets re-extracted in lysis buffer containing different salt concentrations (0.2 M, 0.5 M and 1 M KCl) and again separated in soluble (Sup 2) and unsoluble (Pel) fraction.

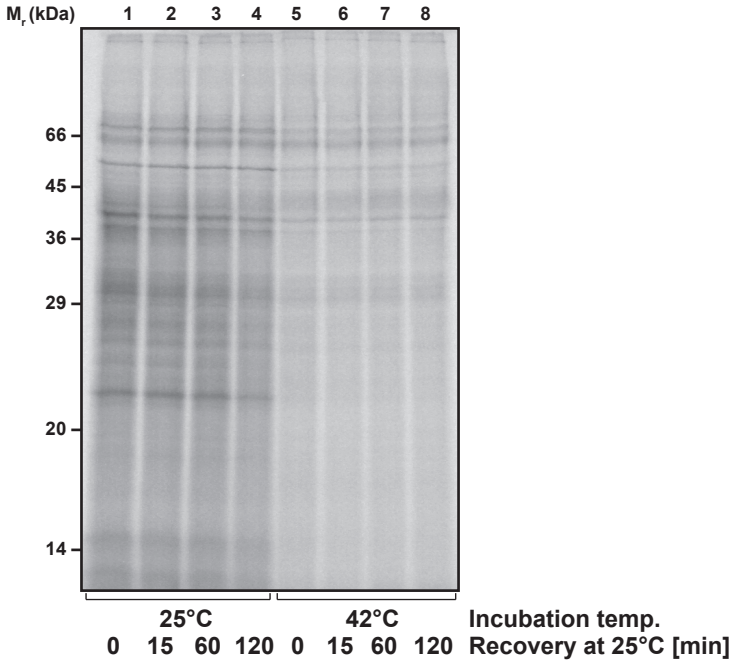

Fig. S5. Recovery of mitochondrial translation.
Fresh isolated energized mitochondria were incubated at 25°C and 42°C for 20 min and afterwards recovered for up to 2 h at 25°C. Newly synthesized proteins were radioactively labeled with [^{35}S]–Met/Cys for 45 min at 30°C. Samples were analyzed on a 15% Urea–SDS–gel and autoradiography.

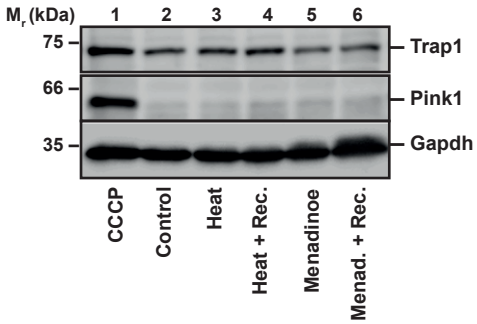

Fig. S6. Stress-induced accumulation of the mitochondrial kinase Pink1.
HeLa wild-type cells were treated for 1 h at 45 °C (heat stress) or 30 min in presence of menadione to induce oxidative stress (high ROS). Were indicated, cells were recovered for 8 h at 37°C. As control, cells were treated with CCCP (carbonyl cyanide m-chlorophenyl hydrazine) for 9 h at 37°C. Total cell extracts were separated by SDS-PAGE and analyzed by Western blot using the indicated antibodies (Gapdh and Trap1 were used as cytosolic and mitochondrial loading controls, respectively).